TRANSFER OF THE *ESCHERICHIA COLI* K12 CHROMOSOME IN THE ABSENCE OF DNA SYNTHESIS

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Several recent reports bearing on the elucidation of the mechanism by which transfer of the chromosome is effected from donor to recipient cell in the *Escherichia coli* K12 mating system have suggested that DNA synthesis plays a role in the process. Jacob *et al* (1963) have proposed a model which explains the transfer process as a result of concomitant replication of the donor chromosome; Bouck and Adelberg (1963) have suggested that the initiation of DNA replication opens the donor chromosome at the F-attachment site, and if conjugation with a recipient occurs, the open chromosome is transferred as soon as the cycle of DNA replication is completed.

The present report presents evidence to show that normal chromosome transfer in one Hfr strain occurs without concomitant DNA synthesis.

MATERIALS AND METHODS

The bacterial strains used were *E. coli* K12 Hfr G6 *his*$^-$; a derivative of it with a partial requirement for thymine, Hfr G6 *his*$^-$ *thy*$^-$; and F$^-$ *pro*$^-$, all from our laboratories; and F$^-$ *arg*$_6^-$, derived from PA265 Maas/Jacob. The symbols correspond to donor (Hfr), recipient (F$^-$), and synthesis of histidine (*his*), thymine (*thy*), arginine-6 (*arg*$_6$), and proline (*pro*).

All cultures were grown in minimal salts-glucose media with the appropriate supplement. Platings for recombinants were made on hard (1.5%) minimal salts-glucose agar; for viable counts on nutrient agar.

Mating was carried out by the membrane filter technique of Matney and

Achenbach (1962). Approximately 5 x 10^7 donor cells and 5 x 10^8 recipient cells were used per filter.

DNA, RNA and protein determinations were carried out on 10 ml aliquots of an incubating culture by the methods of Burton (1956), Visser and Chargaff (1948), and Lowry, et al (1951), respectively.

RESULTS

Incubation of G6 his$^-$ thy$^-$ without histidine results in cessation of DNA synthesis after a 10 - 15% increase (Fig. 1). The same is true for G6 his$^-$. Thus, DNA synthesis by these organisms is routinely sharply curtailed during mating by the filter technique, since the mating plates contain only minimal salts, thiamine, glucose and agar.

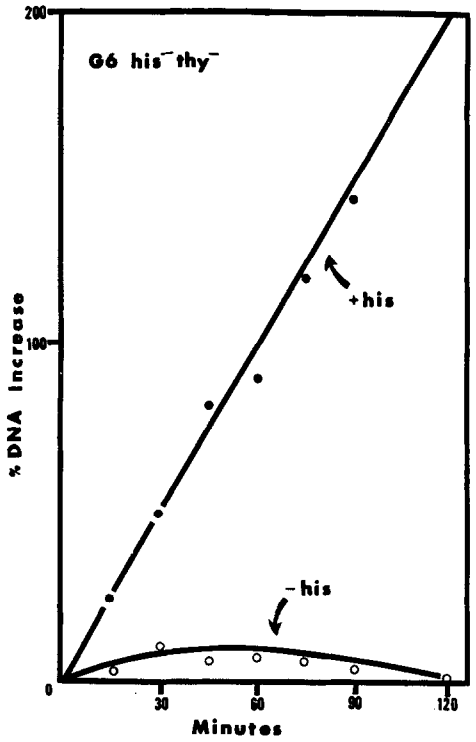

Fig 1

In order to allow completion of residual DNA synthesis before mating, G6 his$^-$ thy$^-$ was incubated without histidine for one or two hours. The cells were then mated with F$^-$ arg$_6^-$. Arg$_6$ is a lead marker (9.5 minutes entry time) in this donor chromosome. The histidine starved cultures

showed entrance kinetics essentially identical to those shown by the normal logarithmic phase cultures and usually gave even somewhat greater total numbers of recombinants (Fig. 2; donor pre-starved for one hour). Similar results were obtained when the histidine-starved donor was mated with F^- $\underline{pro}^-$. $\underline{Pro}$ is a marker about half-way (55 minutes) from the origin.

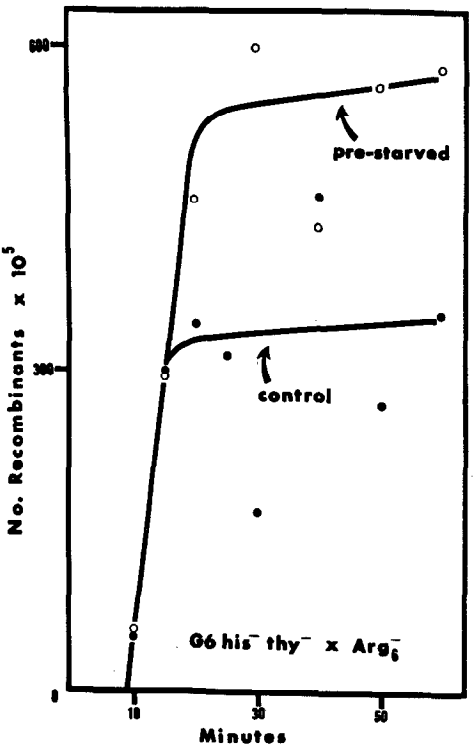

Fig 2

In an attempt to further insure against DNA synthesis during mating, 0.4% phenethyl alcohol (PEA), reported to be a specific inhibitor of DNA synthesis (Berrah and Konetzka, 1962), was added to the mating plates. It was found to decrease the numbers of recombinants formed when either normal or histidine-starved G6 $\underline{his}^-$ $\underline{thy}^-$ was mated to $\underline{arg}_6^-$, but with considerable variability. The extent of the decrease in numbers of recombinants corresponded roughly to the extent of donor killing by PEA observed in a given experiment. The killing ranged from 0% to 90%. Furthermore, PEA was found to drastically inhibit synthesis of RNA and protein as well as synthesis of

DNA by this organism. Therefore, our results obtained with PEA indicate that it cannot be used to provide definitive information in regard to the implication of DNA synthesis in the chromosomal transfer process.

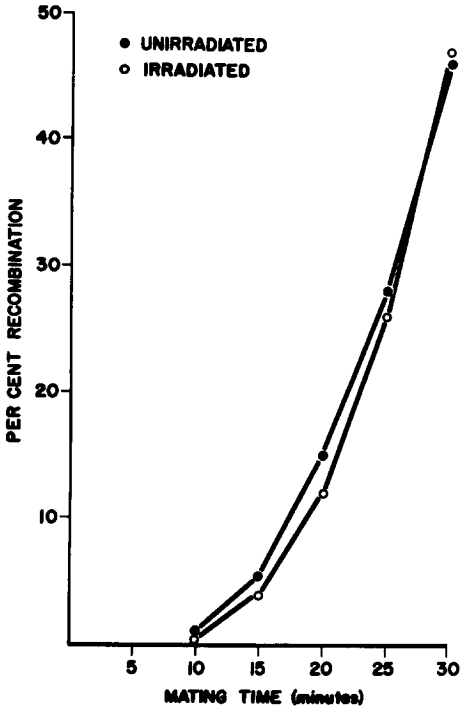

Fig. 3. The per cent of recombination of the arginine-6 locus with irradiated and control E. coli K12 G6 Hfr his^- mated to F^- arg_6^-. The irradiated culture was exposed to 100 ergs per mm^2 ultraviolet light from a General Electric germicidal lamp. Recombination is calculated based on surviving organisms. This dose of UV reduced survival of the G6 donor from 4.0×10^8 cells per ml to 1.8×10^8 cells per ml. At this dose of UV, DNA synthesis is lagged 45 minutes and requires histidine for recovery. No histidine was present during the mating period.

As another means of inhibiting DNA synthesis in donor cells, growing cultures were exposed to ultraviolet light. The survivors of this treatment exhibit a marked lag in DNA synthesis (Kelner, 1953), and cannot recover DNA synthesizing capacity in the absence of RNA and protein synthesis (Harold and Ziporin, 1958; Drakulic and Errera, 1959; and Doudney, 1959). Under the mating conditions used RNA and protein synthesis and, therefore, DNA synthesis also, were prevented in the irradiated donor by the omission of the essential amino acid from the mating plates. However,

the survivors of a UV-irradiated culture of G6 his$^-$ mated immediately following UV exposure showed entrance kinetics of the arginine marker identical to those of a control (Fig. 3).

DISCUSSION

Since Hfr G6 can transfer its chromosome normally while prevented from DNA synthesis by either histidine deprivation or prior exposure to ultraviolet light, it appears that concomitant DNA synthesis is not obligate for the transfer process.

Whether or not completion of a cycle of DNA synthesis is necessary prior to transfer is still not clear. The amount of DNA synthesis (10 - 15%) which occurs during histidine starvation is not enough to account for completion of all of the chromosomes in a random population, yet the numbers of recombinants obtained assures the participation of essentially all donor cells in the mating process. Further investigation of the problem is underway.

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